Reprinted from

BIOMOLECULAR STRUCTURE, CONFORMATION,
FUNCTION AND EVOLUTION

Volume 1

DIFFRACTION AND RELATED STUDIES

by

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PERGAMON PRESS OXFORD and NEW YORK 1980

CRYSTAL AND MOLECULAR STRUCTURE OF SOME NEW PHARMACEUTICALS AND FUNGAL METABOLITES

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IN THIS contribution we would like to present results of the X-ray structure analysis of several biomolecules. These researches were carried out in the Department of Biochemistry, Technical University of Gdansk.

One of the compounds studied is a derivative of erythromycin-A cyclic carbonate. This compound can be obtained from parent antibiotic by reacting it with ethylene carbonate. Erythromycin-A carbonate is the only derivative among many studied modifications, showing higher anti-microbial activity than erythromycin A itself. The carbonate had been synthesized previously in the Eli Lilly Laboratories and a formula was suggested possessing carbonate group connected to carbon atoms C(9) and C(11) (Fig. 1).

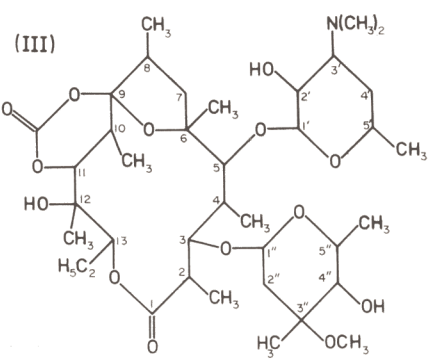

FIG. 1. The formula proposed earlier for erythromycin A carbonate.

Our analysis has been performed with *N*-methyliodide derivative of the compound. Obtained crystallographic data are as follows:

$$a = 14.35 \text{ Å}, b = 14.38 \text{ Å}, c = 10.91 \text{ Å}, \beta = 96.10°, Z = 2, \text{space group: } P2_1$$

The structure was solved by the Patterson and Fourier methods and refined first by block-diagonal and then by full-matrix least-squares procedures. Difference synthesis calculations revealed the presence of one methanol molecule in the crystal structure. This

molecule was also subsequently included in the refinement procedure. From a final difference synthesis map we located all 70 hydrogen atoms, although the positions of some of these atoms were corrected for better geometrical fitting. In the last two cycles of refinement, the hydrogen atoms were included in structure factor calculations, but were not refined.

We found, as shown in Fig. 2, that the carbonate group was connected with carbon atoms C(11) and C(12), instead of with C(9) and C(11) as had been suggested earlier (Fig. 1).

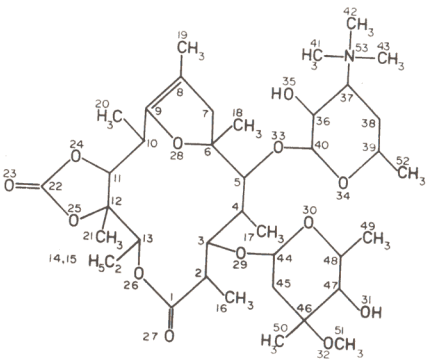

FIG. 2. Formula for the studied derivative of erythromycin A carbonate.

The resulting carbonate ring is thus five-membered and not six-membered. At hemiketal C(9) carbon atom, however, a dehydration resulted leading to the formation of five-membered cyclic enol ether. There are some strains in the ring arising from the fact that this ring is simultaneously a portion of a fourteen-membered macrolide. This is clearly visible, because atoms in the neighbourhood of C(8)–C(9) double bond do not lie in a common plane.

Both sugar moieties in the erythromycin molecule, α-glycosidically bounded cladinose and β-glycosidically bounded desosamine are in chair conformations.

It was recognized that there are only intermolecular hydrogen bonds in the crystal structure of the compound discussed above. One of the hydrogen bonds occurs between the methanol molecule and the iodide anion, the second between cladinose hydroxyl group and the oxygen atom of methanol and the third one between hydroxyl group of desosamine of one molecule and the carbonyl oxygen of macrolide ring of neighbouring molecule (Fig. 3).

Another compound studied in our laboratory is 1-nitro-9(3′-dimethylaminopropylamino) acridine (Fig. 4). The compound was synthesized in our department and introduced into clinical use under the name Ledakrin (World Health Organization adopted as international name—Nitracrine).

This compound exhibits several rather unusual physicochemical as well as biochemical properties. Ledakrin undergoes easily many substitution reactions in the A-ring including the exchange of nitro group. Subsequently, the Ledakrin nitro group is far more susceptible to reduction (also polarigraphic ones) than such a group in other isomers (2-, 3-, 4-nitro). Also the kinetics of hydrolysis of alkylamine substituent on C(9) is quite different from that in the isomers—the Ledakrin hydrolysis rate is higher in alkaline than in acidic

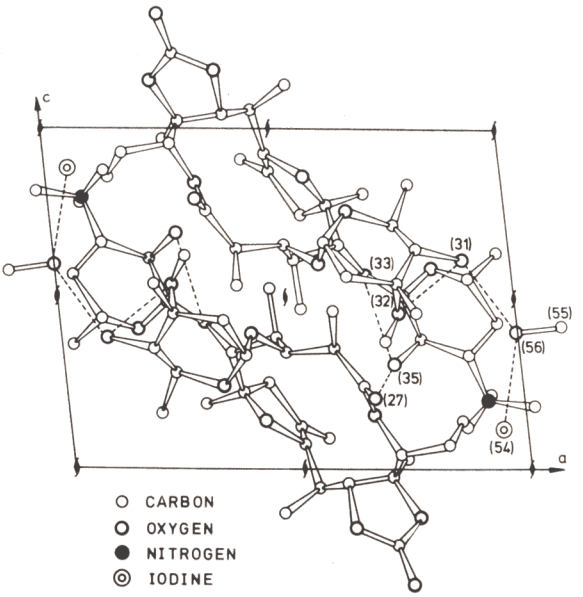

FIG. 3. The *b*-axis projection of the crystal structure of erythromycin derivative.

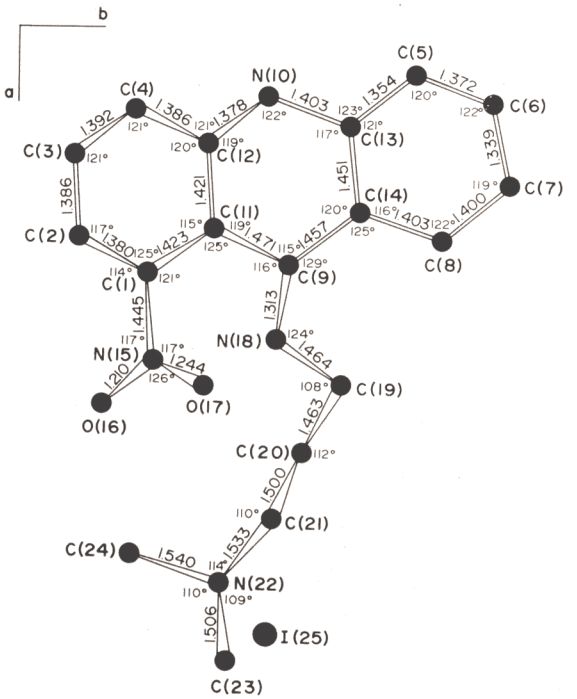

FIG. 4. Atom numbering in Ledakrin with bond lengths and angles for monohydroiodide form.

solutions. Ledakrin exhibits in *in vitro* studies with cell free systems no higher biological activity than other nitroacridines, but in studies with living cells its activity is much higher than that of the other isomers. It has been stated that Ledakrin anti-tumour activity relies mainly on a cross-linking of both DNA threads by its metabolite. So Ledakrin is a latent anti-tumour drug. By this fact one can explain its rather low destructive influence on the blood circulation system.

We have analysed Ledakrin in the free base form as well as in the form of monohydroiodide and dihydrochloride. The dihydrochloride form is used as a medicine.

The crystal data for the three forms of Ledakrin are presented below:

Free base	Monohydroiodide	Dihydrochloride
$a = 14.474$ Å	$a = 24.53$ Å	$a = 13.284$ Å
$b = 10.715$ Å	$b = 19.94$ Å	$b = 11.074$ Å
$c = 12.257$ Å	$c = 7.83$ Å	$c = 15.880$ Å
$\alpha = 110.41°$	$Z = 8$	$\beta = 119.44°$
$\beta = 90.90°$	Space group: Pbca	$Z = 4$
$\gamma = 107.78°$		Space group: $P2_1/c$
$Z = 4$		
Space group: $P\bar{1}$		

The structure of the monohydroiodide form has been solved by the heavy-atom method; the free base as well as the dihydrochloride form were solved by direct methods by MULTAN program.

We have concluded that the acridine core is not planar, but folded along the C(9)–N(10) bond, so that the molecule looks like a butterfly (Fig. 5). The dihedral angle between planes of both side rings is greater than 20°.

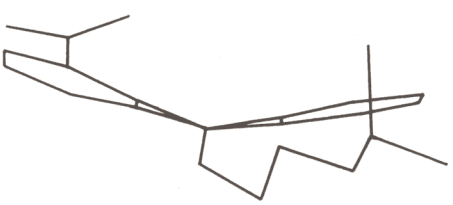

FIG. 5. Ledakrin molecule viewed along C(9)–N(10) bond.

The Ledakrin nitro group is twisted around the C(1)–N(15) bond by about 60°. Thus significant deviation from planarity is obviously a result of strong steric interactions between two bulky substituents in the *peri* position, namely the nitro group on C(1) and the alkylamine group on C(9). This twisting of the nitro group results in its deconjugation with the aromatic system and in the lack of electronic deficit compensation. Hence, the nitro group is more susceptible to reduction than in other isomers.

The sum of valency angles around C(9) is almost equal to 360°; thus the following atoms, C(9), N(18), C(19), C(11), and C(14), lie on a common plane. Due to the folding of the

Ledakrin molecule, the distance between N(18) and the nitro group is increased. The central ring of acridine, however, is not planar, but has a "boat"-like conformation. This diminishes its aromaticity and π-electrons show a tendency to localization on N(10) nitrogen atom and C(9)–N(18) bond. In the free base and monohydroiodide, where the system of vinylogue amidine is not protonated, the molecule represents by itself a more stable tautomer—iminoacridane, with protonated N(10) nitrogen atom (Fig. 6).

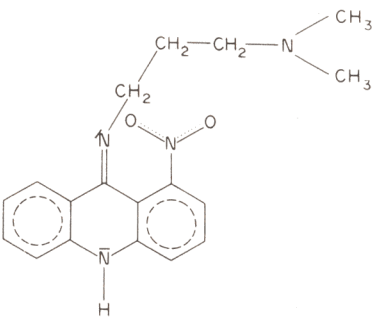

FIG. 6. Iminoacridane tautomer of Ledakrin.

Comparison of the bond lengths in the central acridine ring and the C(9)–N(18) bond shows that in dihydrochloride also the electronic structure is similar to the limiting imino-acridane form.

The presence of double imino bond C(9)–N(18) explains the resistance of Ledakrin against hydrolysis in alkaline solutions, comparing with the other 9-aminoacridines, obviously possessing the aminoacridine tautomeric form. An internal valency angle at the ring nitrogen atom N(10) is always greater than 120° by about 2–3°, that is characteristic for nitrogen bonded with hydrogen.

In all the three forms of Ledakrin the aliphatic chain is unstrained and all bonds have a *syn*- or *anti*-clinal conformation. In spite of the C(9)–N(18) double bond the molecule is obviously an isomer of E or *trans* type.

Ledakrin is of similar conformation to the free base as well as the mono- or diprotonated form. As already mentioned, this specific conformation results from strong steric intra-molecular interactions. Therefore, it is almost certain that in solution Ledakrin will adopt the same conformation and imino tautomeric form. This is also confirmed by kinetic studies and inspection of n.m.r. spectra with use of paramagnetic shift reagents.

The crystal structure of Ledakrin dihydrochloride is quite interesting (Fig. 7). The oblique stacking can be observed and there is a methanol molecule at the centre of symmetry in a "hydrophilic" zone between the acridine molecules. With respect to the orientation of the methanol molecule, some disorder is observed. The methanol molecule is surrounded by a cage consisting of eight chloride anions, connected with charged N(10) and N(22) nitrogen atoms by hydrogen bonds (Fig. 8). Thus the structure is a kind of clathrate because there are not any specific interactions between the methanol and chloride ions.

The monohydroiodide is the only studied crystal derivative of acridine, which shows the "head-to-head" and not "head-to-tail" stacking.

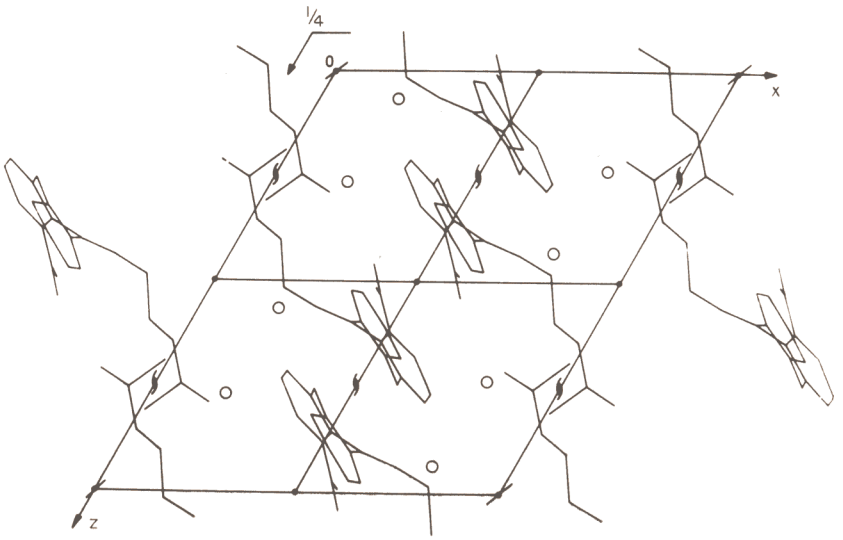

FIG. 7. Packing of molecules of Ledakrin dihydrochloride along the *b*-axis.

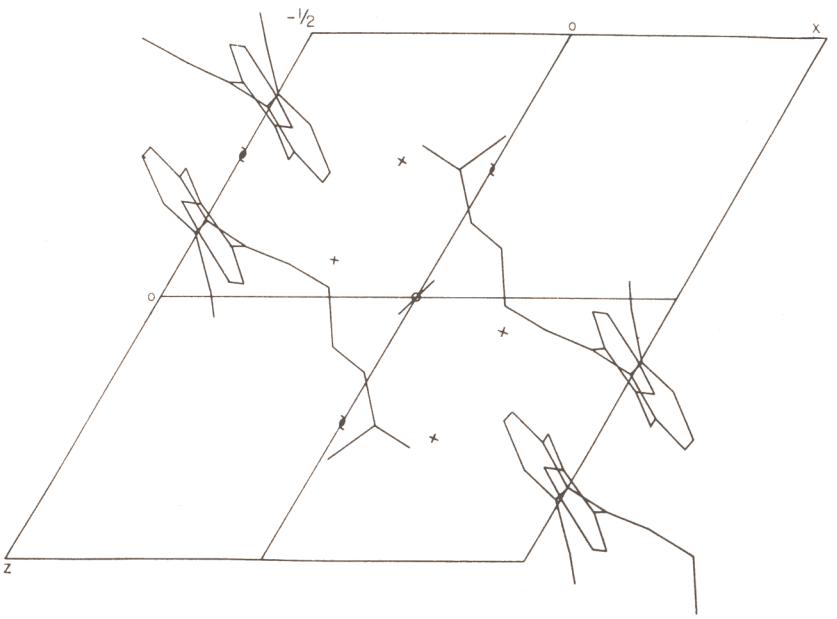

FIG. 8. Surrounding of methanol (solvent molecule) in the crystal structure of Ledakrin dihydrochloride.

We have also investigated the structures of two metabolites of *Lactarius rufus* L.—basidimycetes-lactarorufin B and isolactarorufin. Milky cellular juice isolated from *L. rufus* exhibits certain antibiotic properties. The two compounds were studied in the form of *p*-bromobenzoic esters. The crystal data are summarized below:

Lactarorufin B *p*-bromobenzoate:

$a = 16.55$ Å
$b = 6.75$ Å
$c = 20.07$ Å
$\beta = 111.7$
$Z = 4$
Space group: C2

Isolactarorufin *p*-bromobenzoate:

$a = 15.58$ Å
$c = 7.73$
$Z = 3$
Space group: $P3_1$

Figure 9 shows the *b*-axis projection of the structure of lactarorufin B. The observed structure is slightly different from that previously proposed on the basis of chemical and spectral

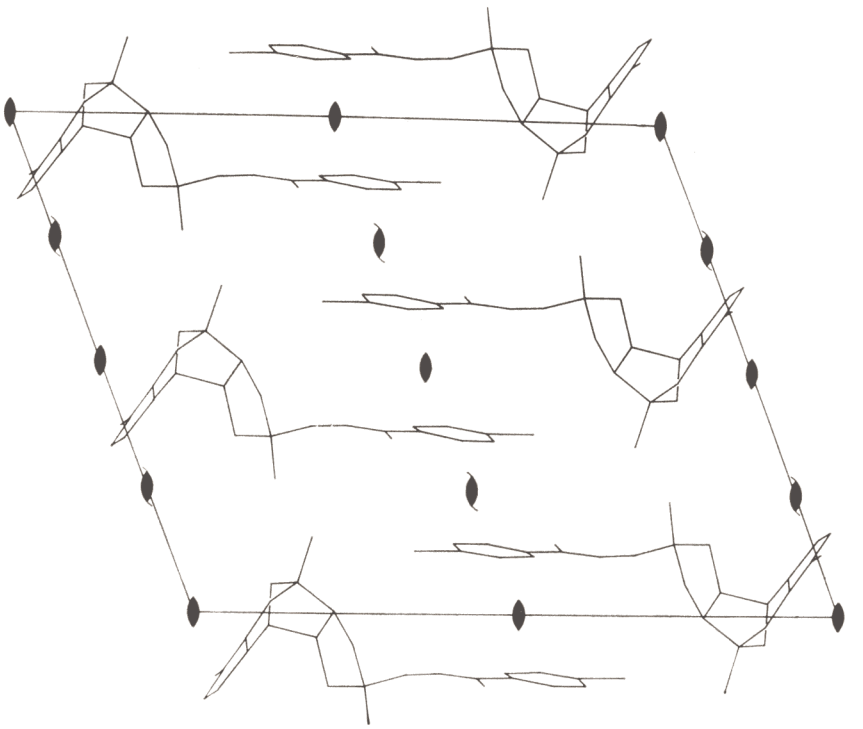

FIG. 9. The *b*-axis projection of the crystal structure of lactarorufin B.

investigations (Fig. 10). The carbonyl oxygen atom of lactone D-ring occurs at C(5) and not at C(13) carbon atom. The lactone D-ring has double bond conjugated with carbonyl group and adopts a planar conformation. Both other five-membered rings, A and B, adopt envelope conformation. The six-membered C-ring has a distorted half-chair conformation. The junction of A-, B- and C-rings is *cis–syn–cis* (Fig. 11).

Figure 12 shows the *c*-axis projection of isolactarorufin structure. The intermolecular hydrogen bond between the lactone oxygen and the hydroxyl group at C(3) forms a helicoidal system along the 3_1 axis.

FIG. 10. The formula of lactarorufin B *p*-bromobenzoate.

FIG. 11. The conformation of lactarorufin B molecule.

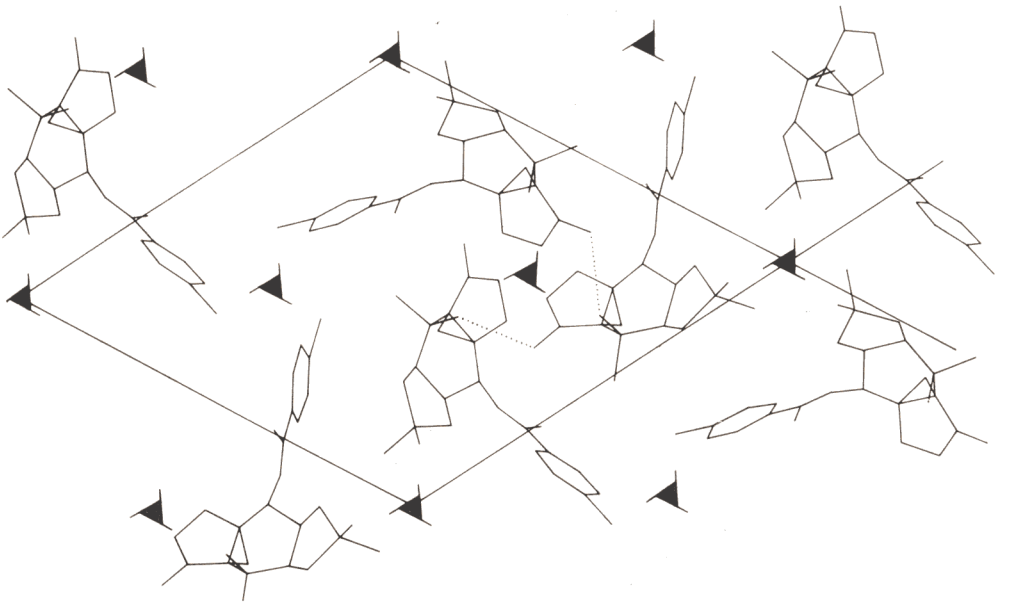

FIG. 12. The crystal structure of isolactarorufin viewed down the *c*-axis.

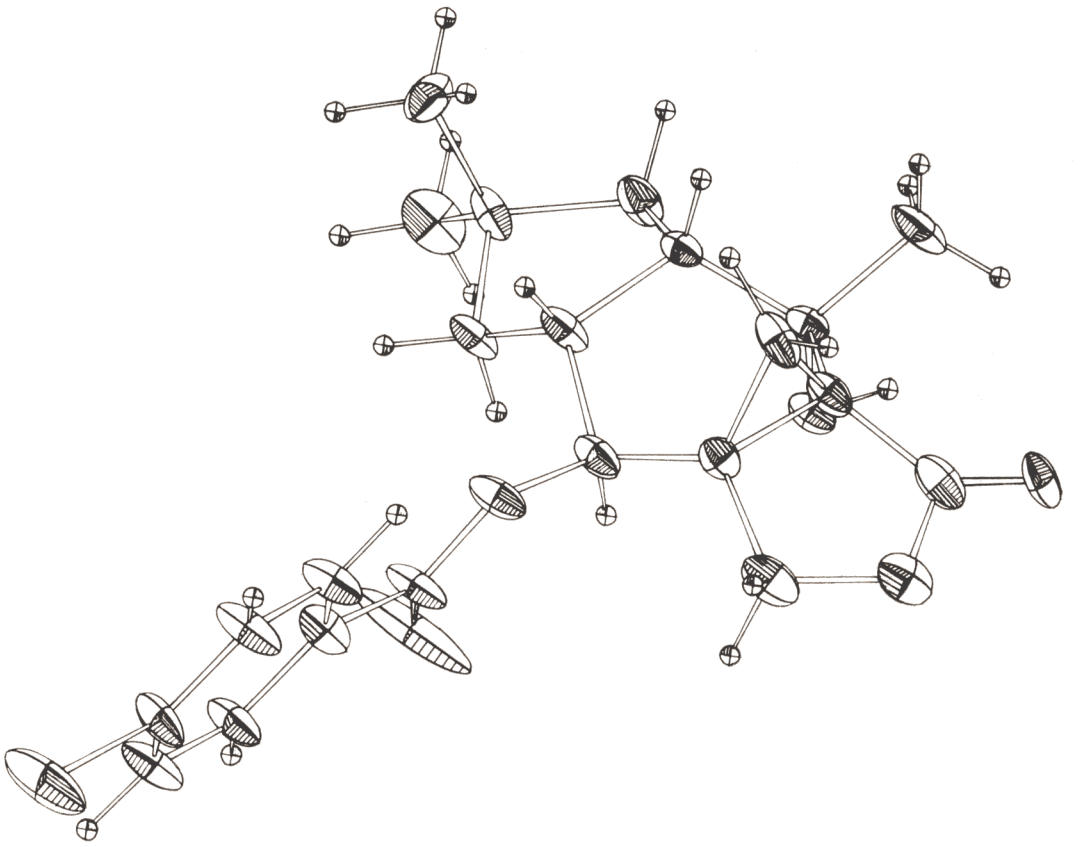

FIG. 13. The conformation of isolactarorufin molecule.

As a result of the presence of cyclopropane ring, the conformation of the attached six-membered ring is boat-like (Fig. 13).

The comparison of results obtained for both lactarorufins may help to clarify the last stages of their biosynthesis.

Acknowledgement. The authors gratefully acknowledge the financial support of these studies by the Institute of Organic Chemistry and the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences.

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